

THIOXAZINOQUINOLONES AS ANTIVIRAL AGENTS

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of the following provisional application: U.S.
5 Serial No: 60/268,302, filed February 13, 2001, under 35 USC 119(e)(i), which is
incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

10 The present invention provides thioxazinoquinolone derivatives having a ring
connecting position 4 (N-4) and position 11 (C-11), and more specifically, provides
compounds of formula (I) described herein below. These compounds are useful as antiviral
agents, in particular, as agents against viruses of the herpes family .

BACKGROUND OF THE INVENTION

15 The herpesviruses comprise a large family of double stranded DNA viruses. They
are also a source of the most common viral illnesses in man. Eight of the herpes viruses,
herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV),
human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and human herpes viruses 6,
7, and 8 (HHV-6, HHV-7, and (HHV-8), have been shown to infect humans.

20 HSV-1 and HSV-2 cause herpetic lesions on the lips and genitals, respectively.
They also occasionally cause infections of the eye and encephalitis. HCMV causes birth
defects in infants and a variety of diseases in immunocompromised patients such as
retinitis, pneumonia, and gastrointestinal disease. VZV is the causative agent of chicken
pox and shingles. EBV causes infectious mononucleosis. It can also cause lymphomas in
25 immunocompromised patients and has been associated with Burkitt's lymphoma,
nasopharyngeal carcinoma, and Hodgkin's disease. HHV-6 is the causative agent of roseola
and may be associated with multiple sclerosis and chronic fatigue syndrome. HHV-7
disease association is unclear, but it may be involved in some cases of roseola. HHV-8 has
been associated with Karposi's sarcoma, body cavity based lymphomas, and multiple
30 myeloma.

Due to the unique position of the chloro substituent on the N-phenylmethyl of
formula I described herein below, compounds of the present invention demonstrate

unexpected activity against the above reference herpesviral infections, particularly, human cytomegaloviral infection.

INFORMATION DISCLOSURE

5 US Patent 5,792,774 discloses oxazino 1,4-dihydro-4-oxoquinolines useful for the treatment of a large number of diseases modulated by tissue necrosis factor (TNF) or phosphodiesterase IV, including cytomegalovirus (CMV) infections.

US Patent 4,847,373 discloses 1,8-bridged 4-quinoline-3-carboxylic acids useful as antibacterial agents.

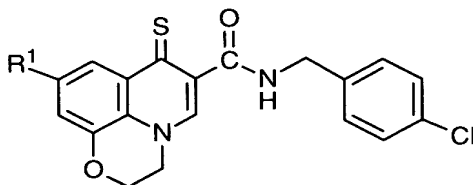
10 US Patent 5,583,135 discloses heterotricyclic derivatives having a strong immunomodulating activity, anti-inflammatory activity and anti-cancer activity.

The abstract of Japanese Patent JP 10324631-A discloses IgE antibody production inhibitor comprise a pyrido(1,2,3-de)1,4-benzoxazine or a pyrido (1,2,3,-de)-1,4-benzothiazine derivative.

15 PCT patent application, PCT/US00/21985 discloses oxazinoquinolones useful for the treatment of viral infections.

SUMMARY OF THE INVENTION

The present invention provides a compound of formula I,



I

wherein R¹ is C₁₋₆ alkyl, optionally substituted with -OH, -OC₁₋₄ alkyl or het;

wherein C₁₋₆ alkyl is optionally partially unsaturated;

wherein het is a radical of a five- or six-membered heterocyclic ring having one or two

25 heteroatoms selected from the group consisting of oxygen, sulfur and N; or a

pharmaceutically acceptable salt, racemate, solvate, tautomer, optical isomer or prodrug derivative thereof.

In another aspect, the present invention also provides:

a pharmaceutical composition comprising a compound of formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier (the composition preferably comprises an effective antiviral amount of the compound or salt),

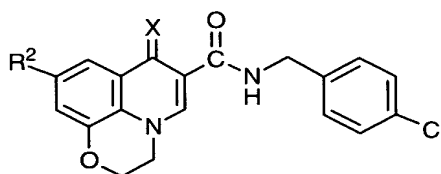
a method of treating or preventing a herpesviral infection, comprising administering
5 to a mammal (e.g. a human) in need of such treatment, a compound of formula (I) or a pharmaceutically acceptable salt thereof,

a method for inhibiting a viral DNA polymerase, comprising contacting the polymerase with an effective inhibitory amount of a compound of claim 1, or a pharmaceutically acceptable salt thereof,

10 a compound of formula I or a pharmaceutically acceptable salt thereof for use in medical treatment or prevention of a herpesviral infection in a mammal.

The invention also provides novel intermediates and processes disclosed herein that are useful for preparing compounds of formula I.

The invention also provides novel intermediates of formula II,



II

15 wherein X is O or S; and R² is optionally partially unsaturated C₁₋₆ alkyl substituted with O-TIPS.

The invention further provides processes disclosed herein that are useful for
20 preparing compounds of formula I.

DETAILED DESCRIPTION OF THE INVENTION

For the purpose of the present invention, the carbon atom content of various hydrocarbon-containing moieties is indicated by a prefix designating the minimum and
25 maximum number of carbon atoms in the moiety, i.e., the prefix C_{i-j} indicates a moiety of the integer "i" to the integer "j" carbon atoms, inclusive. Thus, for example, (C₁₋₃)alkyl refers to alkyl of one to three carbon atoms, inclusive, or methyl, ethyl, propyl and isopropyl, straight and branched forms thereof.

The compounds of the present invention are named according to the IUPAC or
30 CAS nomenclature system.

The term "C₁₋₆" alkyl refers to an alkyl group having one to six carbon atoms such as, for example, methyl, ethyl, propyl, butyl, pentyl, hexyl, and their isomeric forms thereof.

The term "partially unsaturated" refers to an alkyl group having one or more double, or triple bonds.

5 The term O-TIPS refers to triisopropylsilyloxy.

A 5- or 6-membered heterocyclic ring includes pyridinyl, morpholinyl, thiomorpholinyl, piperazinyl, imidazolyl, or pyrrolyl.

Compounds of the present invention may be in a form of pharmaceutically acceptable salts.

10 "Pharmaceutically acceptable salts" refers to those salts which possess the biological effectiveness and properties of the parent compound and which are not biologically or otherwise undesirable.

Compounds of the invention may have a chiral center and be isolated in optically active and racemic forms. It is to be understood that the present invention encompasses any
15 racemic, optically-active, polymorphic, tautomeric, or stereoisomeric form, or mixture thereof.

Specific and preferred values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents

20 Specifically, C₁₋₆ alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, hexyl, or heptyl; het can be pyrrolidino, piperidino, morpholino, thiomorpholino, or piperazine.

A specific value for R¹ is C₁₋₆ alkyl, which may be partially unsaturated and is optionally substituted by hydroxy or het.

25 A specific value for R¹ is propyl.

A specific value for R¹ is 3-hydroxypropyl.

A specific value for R¹ is 3-hydroxy-1-propynyl.

A specific value for R¹ is 4-morpholinylmethyl.

Examples of the present invention are:

- 30 a. N-(4-Chlorobenzyl)-9-(4-morpholinylmethyl)-7-thioxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide,
b. N-(4-Chlorobenzyl)-9-(3-hydroxy-1-propynyl)-7-thioxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide,

- c. N-(4-Chlorobenzyl)-9-(3-hydroxypropyl)-7-thioxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide, or
- d. N-(4-Chlorobenzyl)-7-thioxo-9-propyl-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide.

5 The following Charts A-B describe the preparation of the compounds of formula I of the present invention. All of the starting materials are prepared by procedures described in these charts, by procedures well known to one of ordinary skill in organic chemistry or can be obtained commercially. All of the final compounds of the present invention are prepared by procedures described in these charts or by procedures analogous thereto, which

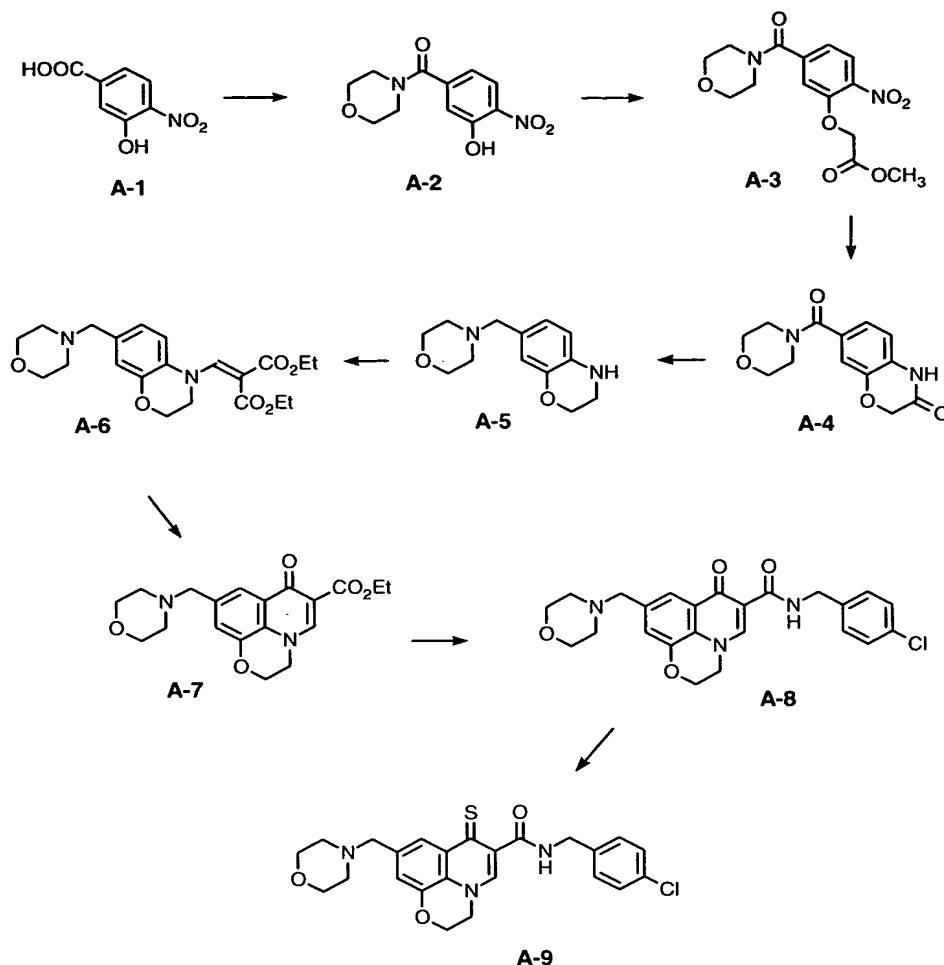
10 would be well known to one of ordinary skill in organic chemistry. All of the variables used in the charts are as defined below or as in the claims.

In Chart A, acid **A-1**, 3-hydroxy-4-nitrobenzoic acid, is reacted with thionyl chloride to give the corresponding acid chloride, which is then treated with morpholine to provide amide **A-2**. Alkylation of the phenolic hydroxyl group is accomplished using methyl

15 bromoacetate and potassium carbonate in refluxing acetone to give **A-3**. Reduction of the nitro group using hydrogen gas and catalytic palladium, followed by thermal cyclization of the intermediate amino ester, provides lactam **A-4**. Reduction of the lactam with lithium aluminum hydride affords amine **A-5**. Reaction of the amine with diethyl ethoxymethylenemalonate provides **A-6**, which is heated with polyphosphoric acid or a

20 solution of phosphorus pentoxide in methanesulfonic acid, effecting cyclization to tricyclic ester **A-7**. Aminolysis of the ester using *p*-chlorobenzylamine at 150 °C provides amide **A-8**. Finally, **A-8** is converted to the desired thioxazinoquinolone by reacting **A-9** with a Lawesson's reagent in the presence of KHMDS in refluxing toluene.

CHART A



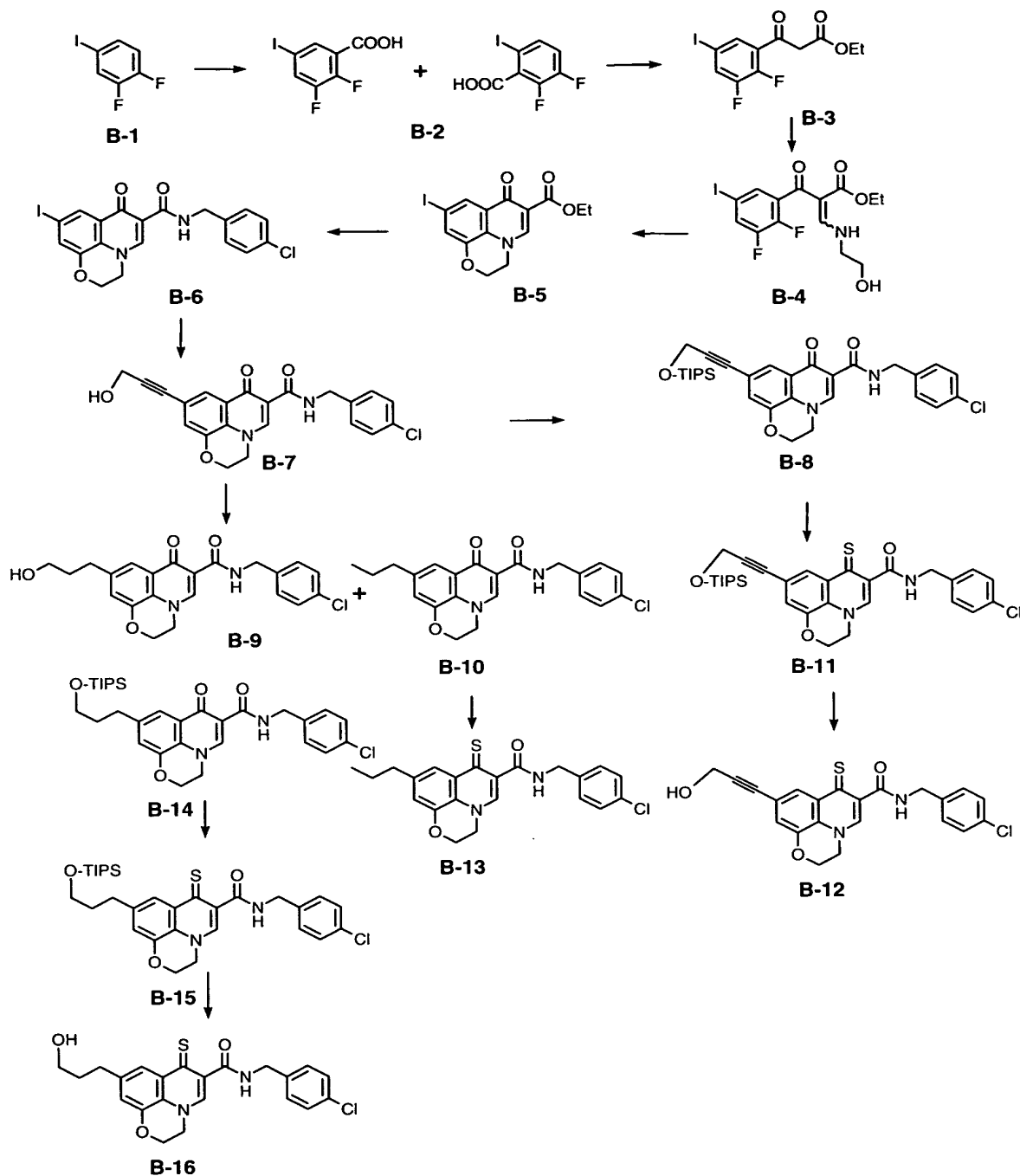
In Chart B, deprotonation of **B-1**, 3,4-difluoriodobenzene, with lithium
 5 diisopropylamide in tetrahydrofuran, followed by quenching of the anion with carbon
 dioxide, provides regioisomeric mixture of acids **B-2**. Activation of the acid mixture with
 carbonyldiimidazole, followed by reaction of the imidazolide with ethyl trimethylsilyl
 malonate in the presence of base affords the single β -ketoester **B-3**. Sequential treatment of
 the β -ketoester with triethyl orthoformate and ethanolamine affords compound **B-4**, which
 10 is cyclized using potassium carbonate in DMF to provide tricyclic ester **B-5**.

Aminolysis of the ethyl ester using *p*-chlorobenzylamine furnishes amide **B-6**,
 which is converted to alkynol derivative **B-7** by palladium (II) catalyzed coupling of the
 iodide with propargyl alcohol. Catalytic hydrogenation of the alkyne using hydrogen gas
 and platinum on carbon provides hydroxypropyl compound **B-9** and *n*-propyl analogue
 15 **B-10**. Alternatively, **B-7** is reacted in DMF with TIPSCl in the presence of imidazole to

- afford **B-8** (See Wuts, P. G. *Protecting Groups in Organic Chemistry* 1999, 123). Reacting **B-8** with Lawesson's reagent in the presence of KHMDS in refluxing toluene provides the thioketone **B-11**. The protected alcohol **B-11** is then treated with $\text{Bu}_4\text{N}^+\text{F}^-$ in THF affording the hydroxyl compound **B-12** (See Wuts, P. G. *Protecting Groups in Organic*
- 5 *Chemistry* 1999, 124). **B-10** is converted to the desired thioxazinoquinolone utilizing Lawesson's reagent in the presence of KHMDS in refluxing toluene. Utilizing similar chemistry described for the preparation of **B-12**, **B-9** is converted to **B-16**.

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CHART B



It will be apparent to those skilled in the art that the described synthetic procedures are merely representative in nature and alternative synthetic processes are known to one of ordinary skill in organic chemistry.

The compounds of the present invention and pharmaceutically acceptable salts thereof are useful as antiviral agents. Thus, these compounds are useful to combat viral infections in animals, including man. Specifically, these compounds have anti-viral activity

against the herpes virus, cytomegalovirus (CMV). These compounds are also active against other herpes viruses, such as the varicella zoster virus, the Epstein-Barr virus, the herpes simplex virus, and the human herpes virus type 8 (HHV-8).

Also, while many of the compounds of the present invention have shown activity
5 against the CMV polymerase, these compounds may be active against the cytomegalovirus by this or other mechanisms of action. Thus, the description below of these compounds' activity against the CMV polymerase is not meant to limit the present invention to a specific mechanism of action.

The compounds of the present invention have shown activity in one or more of the
10 assays described below. All of these assays are indicative of a compound's activity and thus of its use as an anti-viral agent.

The HCMV polymerase assay is performed using a scintillation proximity assay (SPA) as described in several references, such as N.D. Cook, et al., Pharmaceutical Manufacturing International, pages 49-53 (1992); K. Takeuchi, Laboratory Practice,
15 September issue (1992); US Patent No. 4,568,649 (1986); which are incorporated by reference herein. Reactions are performed in 96-well plates. The assay is conducted in 100 μ l volume with 5.4 mM HEPES (pH 7.5), 11.7 mM KCl, 4.5 mM MgCl₂, 0.36 mg/ml BSA, and 90 nM ³H-dTTP. Assays are run with and without CHAPS, (3-[(3-Cholamidopropyl)-dimethylammonio]-1-propane-sulfonate) at a final concentration of 2
20 mM. HCMV polymerase is diluted in enzyme dilution buffer containing 50% glycerol, 250 mM NaCl, 10 mM HEPES (pH 7.5), 100 μ g/ml BSA, and 0.01% sodium azide. The HCMV polymerase, which is expressed in recombinant baculovirus-infected SF-9 cells and purified according to literature procedures, is added at 10% (or 10 μ l) of the final reaction volume, i.e., 100 μ l. Compounds are diluted in 50% DMSO and 10 μ l are added to each
25 well. Control wells contain an equivalent concentration of DMSO. Unless noted otherwise, reactions are initiated via the addition of 6 nM biotinylated poly(dA)-oligo(dT) template/primer to reaction mixtures containing the enzyme, substrate, and compounds of interest. Plates are incubated in a 25 C or 37 C H₂O bath and terminated via the addition of 40 μ l/reaction of 0.5 M EDTA (pH 8) per well. Reactions are terminated within the time-
30 frame during which substrate incorporation is linear and varied depending upon the enzyme and conditions used, i.e., 30 min. for HCMV polymerase. Ten μ l of streptavidin-SPA beads (20 mg/ml in PBS/10% glycerol) are added following termination of the reaction. Plates are incubated 10 min. at 37 C, then equilibrated to room temperature, and counted on a

Packard Topcount. Linear regressions are performed and IC₅₀'s are calculated using computer software.

A modified version of the above HCMV polymerase assay is performed as described above, but with the following changes: Compounds are diluted in 100% DMSO until final dilution into assay buffer. In the previous assay, compounds are diluted in 50% DMSO. 4.5 mM dithiothreitol (DTT) is added to the polymerase buffer. Also, a different lot of CMV polymerase is used, which appears to be more active resulting in a more rapid polymerase reaction. Results of the testing of compounds of the present invention in this assay are shown in Tables 1 below. Other viral polymerase assays are performed using procedures similar to those described above.

These compounds of the present invention are administered in a pharmaceutical composition containing the compound in combination with a suitable excipient, the composition being useful in combating viral infections. Pharmaceutical compositions containing a compound appropriate for antiviral use are prepared by methods and contain excipients which are well known in the art. A generally recognized compendium of such methods and ingredients is Remington's Pharmaceutical Sciences by E.W. Martin (Mark Publ. Co., 15th Ed., 1975), which is hereby incorporated by reference herein.

The compounds of the present invention are administered parenterally (for example, by intravenous, intraperitoneal or intramuscular injection), topically, orally, or rectally, depending on whether the preparation is used to treat internal or external viral infections.

For internal infections, the compositions are administered orally or parenterally at dose levels, calculated as the free base, of about 0.1 to 300 mg/kg, preferably 1.0 to 30 mg/kg of mammal body weight, and are used in man in a unit dosage form, administered one to four times daily in the amount of 1 to 1000 mg per unit dose.

For parenteral administration or for administration as drops, as for eye infections, the compounds are presented in aqueous solution in a concentration of from about 0.1 to 10%, more preferably about 0.1 to 7%. The solution may contain other ingredients, such as emulsifiers, antioxidants or buffers.

The exact regimen for administration of the compounds and compositions disclosed herein will necessarily be dependent upon the needs of the individual subject being treated, the type of treatment and, of course, the judgment of the attending practitioner.

TABLE 1

polymerase IC ₅₀ (μM)			
Example No.	HCMV	HSV	VZV
1	0.06	--	--

The symbol "--" refers to the data are not determined

The compounds and their preparation of the present invention will be better understood in connection with the following examples, which are intended as an illustration of and not a limitation upon the scope of the invention.

EXAMPLES

Preparation 1 (3-Hydroxy-4-nitrophenyl)(4-morpholinyl)methanone (A-2 of Chart A)

To a stirred mixture of 55.0 g of 3-hydroxy-4-nitrobenzoic acid in 600 ml of dichloromethane is added 35 ml of thionyl chloride and 5.0 ml of DMF. The mixture is stirred and refluxed with exclusion of moisture for 1-2 hours, when it suddenly becomes a clear solution. Refluxing is continued for another hour, then volatiles are removed under reduced pressure. The residual amber oil is dissolved in dichloromethane (200 ml), toluene (200 ml) is added, and the solution again concentrated under reduced pressure. The resulting amber oil is dissolved in 300 ml of dichloromethane, and to this solution, stirred and cooled to 0 °C, is added dropwise 65 ml of morpholine in 200 ml of dichloromethane. The resulting mixture is stirred overnight, then washed with water containing sufficient 6N HCl to render the aqueous phase acidic. The aqueous phase is extracted with one additional portion (100 ml) of dichloromethane, and the combined extracts dried (Na₂SO₄) and concentrated under reduced pressure, affording an orange solid. Recrystallization from 1:3 ethyl acetate in heptane provides 70.34 g of the title compound.

Mp 105.5-106.5 °C

¹H NMR (CDCl₃) δ 3.40, 3.64, 3.79, 7.01, 7.18, 8.18, 10.63 ppm

HRMS (FAB) calcd for C₁₁H₁₂N₂O₅ +H₁ 253.0824, found 253.0832.

Anal. Calcd for C₁₁H₁₂N₂O₅: C, 52.38; H, 4.80; N, 11.11; Found: C, 52.46; H, 4.85; N, 11.11.

Preparation 2 Methyl 2-[5-(4-morpholinylcarbonyl)-2-nitrophenoxy]acetate (A-3 of Chart A)

A mixture of 70.05 g of 3-hydroxy-4-nitrophenyl)(4-morpholinyl)methanone of Preparation 1, 42.2 g of anhydrous potassium carbonate, and 28.9 ml of methyl bromoacetate in 700 ml of acetone is stirred and refluxed for 5 hours. Solvents are then removed under reduced pressure, and the residual solid is partitioned between dichloromethane (300 ml) and water (300 ml) containing sufficient 6N HCl to render the aqueous phase acidic. The aqueous phase is extracted with one additional portion (100 ml) of dichloromethane, and the combined organic phases dried (Na₂SO₄) and concentrated under reduced pressure. Recrystallization of the residue from ethyl acetate and heptane furnishes 85.53 g of the title compound.

Mp 140.5-142 °C

¹H NMR (CDCl₃) δ 3.40, 3.63, 3.78, 3.81, 4.83, 7.04, 7.11, 7.90 ppm

IR 1759, 1637, 1608, 1591, 1525, 1436, 1303, 1213, 1114, 842 cm⁻¹.

Anal. Calcd for C₁₄H₁₆N₂O₇: C, 51.85; H, 4.97; N, 8.64; Found: C, 51.87; H, 5.06; N, 8.61.

Preparation 3 7-(4-Morpholinylcarbonyl)-2H-1,4-benzoxazin-3(4H)-one (A-4 of Chart A)

Methyl 2-[5-(4-morpholinylcarbonyl)-2-nitrophenoxy]acetate of Preparation 2 (85.2 g) is hydrogenated in two batches. Approximately half of the compound is shaken in 600 ml of methanol with 1 g of 5% palladium on charcoal under 20 psi H₂. Shaking under hydrogen is continued for 1 hour after hydrogen uptake ceases. The combined reaction mixtures from two such reductions are refluxed under argon for 5 hours, then cooled to 35 °C and filtered through a pad of Celite. Concentration of the filtrate under reduced pressure provides a yellow-white solid residue, which is recrystallized from 800 ml of acetonitrile to afford 62.32 g of the title compound.

Mp 185.5-187.5 °C

¹H NMR (CDCl₃) δ 3.70, 4.64, 6.89, 7.03, 9.66 ppm

IR 1705, 1617, 1461, 1432, 1284, 1114, 1025, 729 cm⁻¹.

HRMS (FAB) calcd for C₁₃H₁₄N₂O₄ + H₁ 263.1031, found 263.1025.

Anal. Calcd for C₁₃H₁₄N₂O₄: C, 59.54; H, 5.38; N, 10.68.; Found: C, 59.29; H, 5.40; N, 10.63.

Preparation 4 7-(4-Morpholinylmethyl)-3,4-dihydro-2H-1,4-benzoxazine (A-5 of Chart A)

To an ice-cooled suspension of 26.2 g of 7-(4-morpholinylcarbonyl)-2H-1,4-benzoxazin-3(4H)-one of Preparation 3 in 350 ml of dry THF under argon is added in portions 7.6 g of lithium aluminum hydride. The completed mixture is stirred while the temperature of the reaction is allowed to rise slowly to ambient as the ice in the cooling bath melts. The reduction is allowed to proceed overnight, then the reaction is re-cooled to 0 °C and quenched by the cautious sequential addition of 7 ml of water dissolved in 15 ml of THF, 7 ml of 3N NaOH, and 21 ml of water. Dichloromethane (350 ml) and anhydrous Na₂SO₄ (10 g) are added, and the mixture stirred vigorously for 20 minutes. The mixture is then filtered, and the solid washed with several portions of dichloromethane. The filtrate is concentrated under reduced pressure to provide 24.98 g of the title compound.

¹H NMR (CDCl₃) δ 2.42, 3.35, 3.41, 3.68, 4.24, 6.54, 6.69, 6.75 ppm

IR 3370, 2854, 1518, 1350, 1295, 1115, 1006, 863 cm⁻¹.

EI MS m/z 235

Preparation 5 Ethyl 9-(4-morpholinylmethyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate (A-7 of Chart A)

A mixture of 9.37 g of 7-(4-morpholinylmethyl)-3,4-dihydro-2H-1,4-benzoxazine of Preparation 4 and 10.4 g of diethyl ethoxymethylenemalonate is stirred and heated at 140 °C for 2 hours. During this time, ethanol formed in the reaction is removed through a distillation apparatus attached to the reaction flask. The reaction is then cooled under vacuum to afford the enamine intermediate A-6 as a yellow oil. This is dissolved in 20 ml of dichloromethane and added to a stirred solution of 8.5 g of phosphorus pentoxide in 52 ml of methanesulfonic acid. The reaction temperature is kept below 30°C during the addition by controlling the rate of addition and by external cooling of the flask. The completed mixture is heated at 50 °C for 18 hours and then cooled to 0 °C, diluted with dichloromethane and water, and cautiously made alkaline by addition of 10M NaOH. Phases are separated and the aqueous extracted with two additional portions of dichloromethane. The combined extract is dried (Na₂SO₄) and concentrated under reduced pressure to afford a tan solid. Recrystallization from acetonitrile provides 10.0 g of the title compound.

Mp 216-218 °C

¹H NMR (CDCl₃) δ 1.40, 2.44, 3.55, 3.70, 4.27, 4.37, 4.50, 7.28, 7.94, 8.30 ppm

IR 1722, 1689, 1601, 1556, 1504, 1318, 1290, 1247, 1117, 1070, 1032, 880, 807 cm⁻¹.

Anal. Calcd for $C_{19}H_{22}N_2O_5$: C, 63.67; H, 6.19; N, 7.82; Found: C, 63.52; H, 6.21; N, 7.80.

Preparation 6 N-(4-Chlorobenzyl)-9-(4-morpholinylmethyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (A-8 of Chart A)

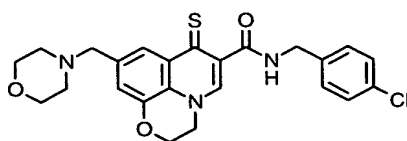
5 A mixture of 4.81 g of ethyl 9-(4-morpholinylmethyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate of Preparation 5 and 7.7 g of *p*-chlorobenzylamine is heated under argon at 150 °C for 18 hours, after which time the bulk of excess amine is distilled off under vacuum. The solid residue is triturated with hexane, filtered and washed with hexane, and dried under vacuum. The resulting solid is
10 chromatographed on silica gel using 2-3% methanol in dichloromethane to provide 5.90 g of the amide as a white solid. Recrystallization from methyl ethyl ketone affords 5.01 g of the title compound.

1H NMR ($CDCl_3$) δ 2.45, 3.58, 3.70, 4.33, 4.51, 4.64, 7.3, 7.94, 8.66, 10.45 ppm

IR (mull) 1651, 1625, 1609, 1558, 1504, 1419, 1316, 1289, 1267, 1116, 1083, 1008, 866,
15 808, 798 cm^{-1} .

Anal. Calcd for $C_{24}H_{24}ClN_3O_4$: C, 63.50; H, 5.33; N, 9.26; Cl, 7.81; Found: C, 63.47; H, 5.37; N, 9.22; Cl, 7.82.

Example 1 N-(4-chlorobenzyl)-9-(4-morpholinylmethyl)-7-thioxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide) (A-9 of Chart A)
20



A mixture of N-(4-Chlorobenzyl)-9-(4-morpholinylmethyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (1.20 g, 2.64 mmol) of preparation 6 is
25 dissolved in dichloroethane (160 mL) followed by the addition of KHMDS (0.5 M, 5.3 mL, 2.64 mmol, 1 equiv.). Then Lawesson's reagent (2.1 g, 5.28 mmol, 2.0 equiv) and toluene (160 mL) is added and the resulting mixture is heated at reflux for 24 h. The reaction is cooled to room temperature, diluted with DCM, washed with water, brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue is purified by silica gel chromatography
30 (heptane/EtOAc 4/1, 1/1, 1/4, 0/1 then DCM/MeOH 19/1) and then repurified by silica gel

chromatography (DCM/MeOH 11/0, 45/1, 9/1) to afford 48 mg of the title compound as a solid.

^1H NMR (TFA_{d1}) δ 9.03, 8.39, 7.88, 7.42, 4.95, 4.81, 4.41, 4.14, 3.84, 3.62.

MS (EI) m/z 469, 438, 436, 245, 243, 218, 217, 216, 160, 125, 56.

5 HRMS (FAB) calcd for $\text{C}_{24}\text{H}_{24}\text{CLN}_3\text{O}_3\text{S} + \text{H}_1$ 470.1305, found 470.1307.

Preparation 7 2,3-Difluoro-5-iodobenzoic acid and 2,3-Difluoro-6-iodobenzoic acid (B-2 of Chart B)

To a stirred solution of 6.7 mL of diisopropylamine in 80 mL of dry THF, blanketed
10 by argon and cooled to -78°C , is added dropwise 27 mL of a 1.6 M solution of *n*-
butyllithium in hexane. The solution is warmed to 0°C and held at that temperature for 10
m, then recooled to -78°C . To the solution is added 4.82 mL of 1,2-difluoro-4-
iodobenzene, and the solution is stirred for 75 m at -78°C before being transferred rapidly
15 via cannula to an excess of solid CO_2 slurried in ether. The mixture is allowed to warm to
room temperature, then is partitioned between ether and dil. NaOH. The aqueous phase is
acidified with dil. HCl and the resulting precipitate extracted with dichloromethane. The
organic phase is dried over MgSO_4 and concentrated under reduced pressure to afford 12.5
g of the mixed acids as a tan oil crystallizing to a solid mass.

^1H NMR (CDCl_3) δ 6.99, 7.61, 7.71, 8.10 ppm.

20

Preparation 8 Ethyl 3-(2,3-difluoro-5-iodophenyl)-3-oxopropanoate (B-3 of Chart B)

To a stirred solution of 11.4 g of difluoroiodobenzoic acid mixture obtained in
Preparation 6 in 40 mL of dry THF under argon is added in portions 7.78 g of
carbonyldiimidazole and 100 mg of DMAP. The solution is stirred for 18 hours at room
25 temperature. Concurrently, 7.48 g of ethyl potassium malonate is suspended in 40 mL of
dry acetonitrile under argon, and to the suspension is added 5.1 mL of
chlorotrimethylsilane. The mixture is stirred for 18 h, then cooled to 0°C for the addition
of 12.5 mL of 1,8-diazabicyclo [5.4.0] undec-7-ene. After 1 h, the acyl imidazolidine
solution is added via cannula, and the resulting mixture is stirred for 1-3 hours. The
30 mixture is then partitioned between ether and excell cold dil. HCl, and the organic phase
washed with dil. HCl and brine, and dried (MgSO_4). Removal of the solvent under reduced
pressure followed by flash chromatography of the residue on silica using 5% ethyl acetate in
heptane provides 3.61 g of the title compound as a white crystalline solid.

^1H NMR (CDCl_3) δ 1.34, 4.28, 5.80, 7.57, 7.95, 12.65 ppm

IR 2989, 1652, 1623, 1490, 1421, 1216, 1037, 960, 800 cm^{-1} .

Preparation 9 Ethyl (E,Z)-2-(2,3-difluoro-5-iodobenzoyl)-3-[(2-hydroxyethyl)amino]-2-propenoate (B-4 of Chart B)

A solution of 1.77 g of ethyl 3-(2,3-difluoro-5-iodophenyl)-3-oxopropanoate of Preparation 7 and 1.3 mL of triethyl orthoformate in 5 mL of acetic anhydride is refluxed for 3 hours, then concentrated *in vacuo* to an oil. This is dissolved in 5 mL of ethanol, and to the stirred solution is added 0.60 mL of ethanolamine. The resulting solution is stirred for 18 hours, then concentrated under reduced pressure. Flash chromatography of the residual oil on silica using 50-75% ethyl acetate in dichloromethane provides 2.06 g of the title compound.

^1H NMR (CDCl_3) δ 0.93, 1.04, 3.3, 3.56, 3.76, 3.9-4.2, 7.37, 7.49, 8.17, 9.75, 10.50 ppm

IR 3400, 2982, 1676, 1635, 1481, 1430, 1313, 1215, 1056 cm^{-1} .

Preparation 10 Ethyl 9-iodo-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate (B-5 of Chart B)

A stirred mixture of 883 mg of ethyl (E,Z)-2-(2,3-difluoro-5-iodobenzoyl)-3-[(2-hydroxyethyl)amino]-2-propenoate of Preparation 8 and 630 mg of powdered potassium carbonate in 10 mL of DMF is heated at 95 °C for 3.5 hours, then cooled and added to 120 mL of water containing 4 mL of 6N HCl. The precipitated product is extracted with chloroform, and the organic phase dried (MgSO_4) and concentrated under reduced pressure. Flash chromatography of the residue on silica using 2% methanol in dichloromethane affords 580 mg of the title compound. An analytical sample may be prepared by recrystallization from acetonitrile.

Mp ~270 °C

^1H NMR ($\text{CDCl}_3 + \text{CD}_3\text{OD}$) δ 1.40, 4.32, 4.37, 4.51, 7.54, 8.31, 8.38

IR 3077, 1718, 1626, 1576, 1552, 1283, 1252, 1158, 800, 719 cm^{-1}

HRMS ($m+H$) 385.9908

Anal. Found for $\text{C}_{14}\text{H}_{12}\text{NO}_4\text{I}$: C, 43.57; H, 3.24; N, 3.63.

Preparation 11 N-(4-Chlorobenzyl)-9-iodo-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (B-6 of Chart B)

A mixture of 575 mg of ethyl 9-iodo-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate of Preparation 9 and 2.0 g of *p*-chlorobenzylamine is heated at 160 °C for 16 hours, then cooled and treated with 20 mL of 1N HCl. After 30 minutes of vigorous stirring, the solid is filtered, washed well with water, and dried *in vacuo*. The solid is further triturated with ether and dichloromethane to afford 563 mg of the title compound as a pale coral solid.

¹H NMR (CDCl₃+CD₃OD+TFA) δ 4.43, 4.53, 4.64, 7.32, 7.63, 8.32, 8.76 ppm

HRMS (m+H) 480.9828

Anal. Found for C₁₉H₁₄N₂O₃ClI: C, 47.55; H, 2.89; N, 5.74.

Preparation 12 N-(4-Chlorobenzyl)-9-(3-hydroxy-1-propynyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (B-7 of Chart B)

To a mixture of 539 mg of N-(4-chlorobenzyl)-9-iodo-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide of Preparation 10, 78 mg of copper (I) iodide, and 30 mg of bis(triphenylphosphine)palladium dichloride in 11 mL of diethylamine, stirred vigorously under argon, is added 82 µL of propargyl alcohol. The mixture is stirred for 18 hours, then diluted with a little methanol and added to 120 mL of stirred water. The precipitated solid is filtered, washed well with water, and dried *in vacuo*. The solid is then dissolved in chloroform-methanol, and the solution is filtered to remove copper salts and then absorbed onto silica gel. Flash chromatography eluting with 2-6% methanol in dichloromethane provides 217 mg of the title compound.

¹H NMR (CDCl₃+CD₃OD) δ 4.39, 4.44, 4.52, 4.63, 7.21, 7.32, 7.91, 8.61 ppm

IR 1643, 1569, 1490, 1450, 1296, 1230, 1087, 1025, 902, 874, 803, 731 cm⁻¹

HRMS (m+H) 409.0952.

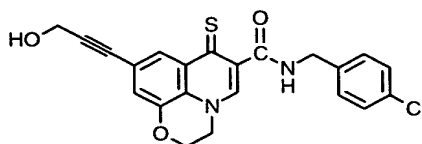
Preparation 13 N-(4-chlorobenzyl)-7-oxo-9-{3-[(triisopropylsilyl)oxy]-1-propynyl}-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (B-8 of Chart B)

Following the procedure described in Wuts, P. G. *Protecting Groups in Organic Chemistry* **1999**, 123-124, reacting N-(4-Chlorobenzyl)-9-(3-hydroxy-1-propynyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide of preparation 12 with TIPSCl in DMF at the presence of imidazole affords the title compound after work-up.

Preparation 14 N-(4-chlorobenzyl)-7-thioxo-9-{3-[(triisopropylsilyl)oxy]-1-propynyl}-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (B-11 of Chart B)

Following the general procedure of Example 1, and making non-critical variations but substituting the products of example 2 (B-8 of Chart B), the title compound is obtained.

Example 2 N-(4-Chlorobenzyl)-9-(3-hydroxy-1-propynyl)-7-thioxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (B-12 of Chart B)



Following the procedure described in Wuts, P. G. *Protecting Groups in Organic Chemistry* **1999**, 123-124, N-(4-Chlorobenzyl)-9-(3-hydroxy-1-propynyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide of example 3 (B-11 of Chart B) is treated with $\text{Bu}_4\text{N}^+\text{F}^-$ in THF affording the title compound.

Preparation 15A and 15B N-(4-Chlorobenzyl)-9-(3-hydroxypropyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (15A, B-9 of Chart B) and N-(4-Chlorobenzyl)-7-oxo-9-propyl-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (15B) (B-10 of Chart B)

A mixture of 50 mg of N-(4-chlorobenzyl)-9-(3-hydroxy-1-propynyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide of Preparation 12 and 20 mg of 5% platinum on carbon in 2 mL of 1:1 THF-methanol is stirred vigorously under 1 atmosphere of hydrogen gas for 3 hours, then filtered through diatomaceous earth. The filtrate is concentrated under reduced pressure, and the residue chromatographed on silica using 2-3% methanol in dichloromethane. Following concentration under reduced pressure, the upper eluting fractions provide 16.5 mg of N-(4-chlorobenzyl)-7-oxo-9-propyl-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (15B); the lower eluting fractions similarly afford 27.5 mg of N-(4-chlorobenzyl)-9-(3-hydroxypropyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (15A).

Physical characteristics for 15A are as follows:

^1H NMR (CDCl_3) δ 1.9, 2.82, 3.62, 4.38, 4.51, 4.63, 7.16, 7.31, 7.82, 8.63

IR 3349, 2937, 1647, 1608, 1557, 1504, 1453, 1286, 1053, 805 cm^{-1}

HRMS (m+H) 413.1266.

Physical characteristics for 15B are as follows:

Mp 205-206 °C;

¹H NMR (CDCl₃) δ 0.94, 1.7, 2.69, 4.31, 4.49, 4.63, 7.11, 7.3, 7.83, 8.64, 10.5 ppm

5 IR 3040, 2929, 1651, 1609, 1558, 1504, 805 cm⁻¹

HRMS (m+H) 397.1316

Anal. Found for C₂₂H₂₁N₂O₃Cl: C, 66.35; H, 5.37; N, 7.03.

10 Preparation 16 N-(4-chlorobenzyl)-7-oxo-9-{3-[(triisopropylsilyl)oxy]propyl}-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (B-14 of Chart B)

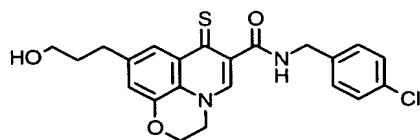
Following the general procedure of Example 2, and making non-critical variations but substituting the products of Preparation 13 (B-9 of Chart B), the title compound is obtained.

15 Preparation 17 N-(4-chlorobenzyl)-7-thioxo-9-{3-[(triisopropylsilyl)oxy]propyl}-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (B-15 of Chart B)

Following the general procedure of Example 1, and making non-critical variations but substituting the products of example 5 (B-14 of Chart B), the title compound is obtained.

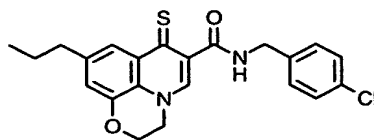
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Example 3 N-(4-Chlorobenzyl)-9-(3-hydroxypropyl)-7-thioxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (B-16 of Chart B)



25 Following the general procedure of Example 4, and making non-critical variations but substituting the products of example 6 (B-15 of Chart B), the title compound is obtained.

Example 4 N-(4-Chlorobenzyl)-7-thioxo-9-propyl-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamide (B-13 of Chart B)



Following the general procedure of Example 1, and making non-critical variations but substituting the products of Preparation 13 (B-10 of Chart B), the title compound is obtained.

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